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THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
310-312 W. MONUMENT STREET
BALTIMORE, MD.

DR. BECKER'S THESIS

PUBLISHED

JOURNAL OF PARASITOLOGY, VOL. IX, 1923, p. 141-152

(No. LX1, Collected Papers)

AMERICAN JOURNAL OF HYGIENE, VOL. III, 1923, p. 462-468

(No. LXIV, Collected Papers)

STUDIES ON SOME PROTOZOAN FLAGELLATES ENTOZOIC IN THE ALIMENTARY TRACTS OF NORTH AMERICAN INSECTS

Dissertation

Submitted to the Director of the School of Hygiene and Public Health of the Johns Hopkins University in Conformity with the requirements for the degree of Doctor of Science in Hygiene.

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June, 1923

Table of Contents

Observations on the Morphology and Life History of Herpetomonas muscae-domesticae in North American muscoid flies . . . . . Page 1-35

Morphological Studies 1

Life History Studies 17

Nomenclature 27

Conclusions 29

Explanation of Plate 31

Bibliography 33

Plate 35

Transmission Experiments on the Specificity of Herpetomonas muscae-domesticae in Muscoid Flies 36-61

Introduction 36

Alexeieff's hypothesis 37

Infections in North American Flies 38

Morphological Identity 39

Methods employed 42

Experimental data 43

Natural cross-infection 54

Discussion 56

Summary 58

Bibliography 60

Studies on the Relationship between Insect Flagellates and Leishmania 62-75

Introduction 62

Previous experiments 63

Experimental data 66

Conclusions and discussions 71

Bibliography 73

Observations on the Morphology and Life Cycle of Crithidia gerris Patton in the water-strider, Gerris remigis Say . 76-104

Introduction 76

Description of the gut of Gerris remigis 77

Technique employed 78

Percentage of Infection 79

Distribution in the host 79

Morphology 80

Manner of multiplication 92

Summary and Conclusions 95

Bibliography 97

Text figures 100

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Observations on the Morphology and Life History of *Herpetomonas muscae-domesticae* in North American Muscoid Flies.

Certain flagellate parasites of insects are of particular interest to investigators because of both their general biological importance and the relationships which they bear to mammalian parasites belonging to the genera *Leishmania* and *Trypanosoma*. Perhaps the commonest and most extensively investigated of all these insect flagellates is *Herpetomonas muscae-domesticae*, a parasite of the common muscoid flies. No attempt will be made to give an extensive review of the work of previous investigators, since it would make this contribution unnecessarily lengthy. The references at the end of this paper and those given by Wenyon (1913) and Brug (1914) should be consulted for previous papers on this and closely related subjects.

The writer has encountered three distinct morphological types of *Herpetomonas* in the muscoid flies of North America. (1) The type which is unmistakably the *Herpetomonas muscae-domesticae* which Prowazek (1904) described, whose distinctive features are strongly staining flagella, each flagellum thickened at the margin of the cell body so as to

form marginal granules, and a large deeply staining parabasal body. This flagellate is almost always found in a state of division, making it appear biflagellated. During the summer of 1922 this species was found to be present in varying percentages in the alimentary canal of Phormia regina, Lucilia sericata, Calliphora erythrocephala, Cochliomyia (Chrysomyia) macellaria, Musca domestica, and Sarcophaga bullata. The Herpetomonas found in these six species of flies were morphologically indistinguishable, and exhibited no more morphological variations in hosts of different species than in hosts of the same species. In a later paper will appear a report on cross-infection experiments which seems to indicate that H. muscae-domesticae is the common parasite of these six species of flies. (2) In Muscina stabulans, Calliphora erythrocephala, and Limosina sp. is a herpetomonad of somewhat smaller size with a flagellum and parabasal body less strongly developed than in H. muscae-domesticae. It corresponds very well with the Crithidia calliphorae of Swellengrebel (1911), although there is no reason why it should be placed in the genus Crithidia. (3) In two individuals of a number of Sarcophaga securifera examined a Herpetomonas quite different from any yet described was found. Its cell body was much rounded, and in the stained preparations showed a deeply staining flagellum, prominent mar-

ginal granules, a deeply staining parabasal body surrounded by a large clear space, and a nucleus with a very small karyosome and a pronounced achromatinic nuclear membrane (Fig. 1). It is the first type of Herpetomonas, however, which is by far the most common. Herpetomonas muscae-domesticae in the host Phormia regina was used for the most part in the investigations recorded in this paper. Of two hundred "wild" flies of this species captured during the month of June, 1922, sixty-six per cent were infected.

The seat of infection is the alimentary canal where the parasites may be distributed from the crop to the rectum. Strickland (1911) made a study of the stages of the parasite in the different regions of the alimentary tract of a species of Lucilia. The fact that infections vary much in their nature should not be overlooked, for in some cases in which the entire intestine swarms with flagellates it is difficult to find any except the long flagellated forms. In another type of infection the crop and anterior end of the midgut may be free from parasites, while the posterior portion of the midgut and the hindgut may contain numberous stumpy flagellates, cysts, and encysting forms. I have rarely found the stumpy and encysting forms in the crop. When herpetomonads are present in the crop, they are generally the long flagellated forms known as "adults" (Figs. 2-15).

A series of fifty "adult" herpetomonads taken ten each from five heavily infected flies gave the average measurements indicated in Table 1. The preparations used in making the measurements were fixed in Schaudinn's fluid (without acetic acid) and stained by Heidenhain's iron-haematoxylin method. A comparison with dried smears stained by the Romanowsky stains showed that the length of the cell body of dried individuals averaged about 5.0 u greater than in the case of those in the "wet mounts."

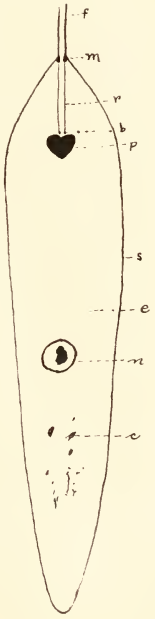
Table 1. Measurements in microns of fifty adult herpetomonads taken at random ten each from five infected flies.

Slide number	Posterior tip to nucleus	Nucleus to marginal granules	Posterior tip to marginal granules	Flagellum	Greatest width	Total length	Range in total length
1	8.6	9.1	17.7	37.4	1.8	55.1	45.4-58.3
2	8.2	8.6	18.8	29.0	2.0	45.8	38.2-49.5
3	6.4	7.7	14.1	34.3	1.8	48.4	36.0-54.0
4	6.8	8.5	15.3	22.7	2.0	38.0	31.0-40.5
5	4.5	7.3	11.8	21.8	1.8	33.6	27.0-36.5
Average	6.9	8.2	15.2	29.0	1.9	44.2	

From this table it can be seen that there is considerable variation in the measurements of this type of the flagellate in various hosts of the same species; e.g., the average total length in one fly was 55.1 u, while in another fly it was 33.6 u. One monster (not considered in above averages) was encountered with a total length of 79.9 u, the body length alone being 40.1 u (Fig. 15). In general the flagellate ranges in length from 25.0 u to 79.9 u, although by far the greater number of individuals measure in the proximity of 45.0 u in length and from 1.5 u to 2.5 u in width.

The accompanying text figure represents diagrammatically the cell structure and the terminology applied to the various parts of the cell. There is a rigid cell body from the anterior end of which emerges a flagellum, or much more frequently the two flagella of the dividing individual, enclosed in a cytoplasmic sheath. Wenyon (1913) considers that the body of the cell is much flattened like a blade of grass. In the average stained preparation where one gets only a side view of the flagellate this appears to be true; but in order to determine whether this was actually the case, I examined a peritrophic membrane filled with the flagellates, many of which had attached themselves by their flagella in

Text Fig. 1, diagrammatic representation of the structure of Herpetomonas muscae-domesticae. f, flagella; m, marginal granules; r, intracytoplasmic flagellum or rhizostyle; b, basal granules; p, parabasal body; s, periplast; e, endoplasm; n, nucleus with nuclear membrane and central karyosome; c, "chromidia."



such a position that their longitudinal axes were parallel to the rays of light passing through the microscope. By focusing up and down upon such individual flagellates it was apparent that their optical cross section was round or oval. The entire cell is enclosed in a thin periplast within which lies the endoplasm and cell organelles, which will be described separately.

Endoplasm. In comparison with Crithidia gerridis the endoplasm is finely granuled. The "chromidia" lying in the endoplasm are smaller than those in Crithidia gerridis, and do not stain intra vitan with Janus green. Swellengrebel (1911) reported that these "chromidia" are composed of volutin. Wenyon found bodies lying in the endoplasm which he thought were bacteria because of their bipolar staining. I have not found these appearances with the frequency with which Wenyon found them, but at times I have found rod-like nuclear fragments in what appear to be degenerating specimens with disrupted nuclei. They stain much heavier, however, than the bacteria on the slide, and are probably not the bodies which Wenyon considered bacteria. In my preparations I can find no evidence of the presence of bacteria in the endoplasm of the cell.

This brings us to the question of a cytostome which Wenyon (1913) has discussed at length. I have carefully searched in the living and stained flagellates for the funnel-like depression surrounding the flagellum which Wenyon describes, but

I can find no evidence for it. It is true that the region of the cell body anterior to the parabasal body is often remarkably transparent, particularly between the two flagella of a dividing cell; but there is no visible aperture through which solid particles of food such as bacteria could be admitted. The periplast enclosing the cell body is in uninterrupted continuity with the flagellar sheath so far as one is able to observe. The anterior end of the cell body of the flagellate with which I worked is not truncated after the fashion of Wenyon's figures. The absence of solid food particles in the endoplasm and the absence of any cytostome suggest that the parasite is saprozoic in its habits.

Flagellum. Prowazek (1904) described the organism as a biflagellate. Almost all subsequent observers (Patton, Porter, Wenyon, Mackinnon, Strickland, etc.) have agreed that the biflagellate appearance is due to the tendency of this organism to be almost constantly in a stage of division, since under some conditions both uniflagellated and biflagellated specimens can be found. The cyst normally contains but the rudiment of one flagellum (Fig. 19), and the trypaniform type (Figs. 17, 18) in most instances is uniflagellated, although dividing specimens with two flagella exist.

The flagellum arises from the basal granule and proceeds anteriorly as a fine line until it reaches the margin

of the cell, where it thickens noticeably to form the marginal granule, or in the dividing forms, the marginal granules (Swellengrebel, 1911) (Figs. 3, 4, 5) which Prowazek termed the (anterior) diplosome. The intracellular portion of the flagellum has often been termed the rhizoplast (Prowazek, Swellengrebel). The extracellular portion of the flagellum is much more sturdy and takes a deeper stain than the intracellular portion.

Basal granule. The basal granules lie immediately anterior to the parabasal body (Figs. 2, 4, 7, 9, etc.), sometimes in contact with it (Fig. 13). Sometimes the intracytoplasmic portion of the flagellum can be traced until it makes a contact with the parabasal body, with no basal granules visible (Figs. 3, 6, 8). In such cases the basal granules must lie either embedded within the parabasal body or in a depression in its anterior surface.

Parabasal body. The parabasal body (blepharoplast of Schaudinn, kinetonucleus of Woodcock) in the adult herpetomonad lies usually from 3.5 u to 4.5 u anterior to the nucleus. It varies somewhat in shape from pear-shaped to bar-shaped. In the dividing specimens it may appear heart-shaped (Fig. 9). In the living cell it is seen with difficulty as a more or less refractile spot. If an infected intestine is teased up in a drop of Janus green made by dissolving one part of the powdered

dye in twenty thousand parts of normal saline solution, the parabasal bodies of the flagellates stain after the typical mitochondrial fashion, which the nucleus does not do. In dried preparations stained by Wright's stain this structure appears wedge-shaped, and sometimes quite elongated as indicated in Fig. 20. This distortion is due to the drying process. A discussion of the parabasal body of these flagellates was included in a previous paper on Crithidia gerridis.

Nucleus. No investigator has considered the cytological details of the nucleus so thoroughly as has Wenyon (1913). He describes the nucleus thus: "There is a fine nuclear membrane enclosing a space, in the center of which lies the deeply staining karyosome." In addition there was observed what the author conceived to be a centriole, which may be within the center of the karyosome (composed of chromatin and plastin), just outside the karyosome, or situated on the nuclear membrane. My own observations agree in the main with those of Wenyon, except in regard to the existence of a visible centriole. It is not to be questioned that chromatin granules which resemble centrioles, may be found in the nucleus, but after studying a large number of dividing specimens I have become convinced that there is no indication of a centriole or other attraction body which per-

forms the function of a centriole during the process of division. Alexeieff (1912) who studied carefully the process of nuclear division in this parasite found no indication of a functioning centriole. The nucleus will be discussed further under the process of division.

Alexeieff describes a rhizostyle or axostyle extending posteriorly from the parabasal body of the haemoflagellates, which is evidenced by either positive or negative staining. Like Wenyon, I am unable to find anything in well-stained preparations like the appearances which Alexeieff figures, but occasionally near the edge of the smear where the specimens have dried somewhat, the dark staining granules in the cytoplasm may accidentally be arranged in rows due to endoplasmic streaming, which was not observed in this species of flagellate, but was observed in Crithidia gerridis.

In addition to the adult herpetomonads just described there are two other stages of this flagellate commonly present, which will be described separately, the trypaniform and the cyst.

The trypaniform stage may be regarded as the transitional stage between the adult flagellate and the cyst form. It is found usually in the posterior part of the midgut and in the rectum. Wenyon has described two methods of encystment, one in which encystment is preceded by the trypaniform stage and one

in which it is preceded by the leptomonas or herpetomonad stage. This is quite markedly true in the case of the small flagellates which I found in Muscina stabulans, but in M. muscae-domesticae I have observed only one manner of encystment, and that from the trypaniform type, which in turn is derived from the adult herpetomonad type. All intermediate stages may be found from that in which the parabasal body approaches the nucleus and becomes in a sense a crithidia (Fig. 16) to the stage in which the parabasal body lies posterior to the nucleus and is structurally a trypanosome, although in neither the crithidia nor trypanosome stage is there an undulating membrane. The body decreases noticeably in length and very slightly in width as the trypaniform condition develops. Usually one flagellum and one nucleus are present, but occasionally dividing forms with a double set of organelles are found.

The parabasal body of the trypaniform type continues to migrate posteriorly, and the cell body becomes shorter, until the organism becomes a small oval body with the mere rudiment of a flagellum (Fig. 18). The extracellular flagellum finally degenerates completely (Fig. 19). The point of entrance of the flagellum into the cyst is plugged by the marginal granule, which continues to stain deeply (Fig. 19).

The intracellular flagellum can be traced to the parabasal body which finally migrates to the extreme posterior end of the cyst, becomes bar-shaped, and stains less deeply than formerly. The nuclear karyosome becomes round or oval, and lies inside a light staining nuclear membrane. The protoplasm nearest the flagellum stains somewhat more lightly than that near the edge of the cyst, but why Strickland (1911) should call this pale staining endoplasm a cytopharynx is not clear.

Method of multiplication. The only method of multiplication observed in this protozoon is binary fission. The processes of copulation, parthenogenesis, etheogenesis, and autogamy, as described by Prowazek are now known to have been based upon misinterpretations. As Kofoid (1916) has said, " . . . we have as yet, except among the volvocidae, which are hardly typical flagellates, little evidence which can be regarded as final that true sexual reproduction occurs at all among flagellates."

Wenyon (1913) and Alexeieff (1912, 1913) made important studies on the process of division of this flagellate, particularly in regard to the nucleus. In my slides fixed in Schaudinn's solution without the acetic acid and stained by the iron-haematoxylin method I have frequently found beautifully stained dividing specimens on which I made

certain observations agreeing except in a few details with those of Alexeieff, but differing somewhat from those of Wenyon, particularly in regard to the presence of a functioning centriole.

The first indication of division is a divided basal granule and a double flagellum (Fig. 3, 4, 5, 6). By far the greater number of flagellates exist in this dividing stage. The doubling of the flagellum comes about, according to Wenyon, Patton, Mackinnon and others, through a new flagellum growing out on intimate contact with the old one, which makes the process difficult to observe. As the two basal granules move away from each other, the parabasal body enlarges somewhat, and commences at the anterior end to split longitudinally (Fig. 9). At this stage it is usually heart-shaped. The splitting continues under the influence of the basal granules until the two new pear-shaped parabasal bodies are entirely separate (Figs. 10, 11). There is no evidence of a mitotic division of the parabasal body such as Rosenbusch (1909) described for trypanosomes and Whitmore (1911) described for Prowazekia asiatica.

The apparent non-uniformity of the appearances in various dividing nuclei is somewhat discouraging to one who would get such clear-cut pictures of the various stages as are possible in metazoan cells. The difficulties are somewhat increased if one postulates the presence of a centriole, as Wenyon

did, and attempts to follow the division in relation to these chromatin fragments resembling centrioles. Wenyon himself expressed the opinion that many of the appearances which he considered centrioles may have been simply separate chromatin bodies. It seems to me that the latter possibility is the correct one, and like Alexeieff, I should place this type of nuclear division in the group with those which have no functioning centriole. Alexeieff calls this type of nuclear division panmitosis, and defines it thus:

"Tout le materiel chromatique (chromatine peripherique ainsi que chromatine caryosomienne) est employe a la formation des chromosomes, de sorte qu'il n'y a ni centrioles ni corps polaires. Le stade de la plaque equatoriale n'est pas net, meme le plus souvent il manque completement. A l'anaphase entre les deux noyaux-fils est etiree une fibre fusoriale."

The resting nucleus may be regarded as a plastin matrix surrounded by a fine achromatinic nuclear membrane in whose center is embedded the karyosome, composed either of one or several chromatin particles (Figs. 2, 3, 4, 21, 22, 23, 24, 25). As was previously noted by Mackinnon (1910) one of the first indications of division in the nucleus is elongation of the karyosome in the longitudinal axis of the cell

(Figs. 26, 27, 28, 29). As the cell body increases in breadth the elongated nucleus is drawn to a position at an angle to its former one (Fig. 30). By this time the nuclear membrane has become indistinguishable from the cytoplasm, and the karyosome begins to resolve itself into a number of discrete granules (Fig. 7, 8, 9, 10, 31, 32, 33). The chromatin mass becomes constricted in the center as the two halves move away from each other. Alexeieff states that the nucleus may be divided into from eight to sixteen chromosomes, but he figures four in each daughter nucleus. I have failed to find more than four of these chromatin fragments or "chromosomes" in each of the daughter nuclei. In some cases where there are two or three granules one or two of them are usually exceptionally large or of a bilobed appearance, suggesting that they are in reality double.

The two daughter nuclei may be connected by an achromatinic filament (Fig. 9), or there may be none present (Figs. 10, 11). This is Wenyon's centrodesmose and Alexeieff's tractus fusorial achromatique. It stains somewhat lighter than nuclear chromatin, and probably represents interzonal nuclear plastin undergoing degenerative changes prior to absorption into the cytoplasm.

The cytoplasm of the mother cell collection passes posteriorly to the posterior end (Fig. 13) and the two individuals remain connected only by the sheath of the flagella (Fig. 14). Very soon they free themselves from each other by their active movements. The chromatin fragments in the nucleus again clump together to form the resting nucleus.

Life History Studies

In his various papers upon Crithidia and Herpetomonas Patton has always emphasized that these flagellates in their life cycle pass through pre-flagellate, flagellate, and post-flagellate stages. It is perhaps due to his influence that it is to be inferred from almost all the publications upon these subjects that this is an obligatory life cycle and that the cyst is the only infective stage. For example, Strickland (1911) and Wenyon (1913) speak of the cyst as the infective form of H. muscae-domesticae. Roubaud (1912) did a series of experiments with Pycnosoma flies and their flagellate parasites from which he concluded that the infection is transmitted from fly to fly by the cysts and not by the flagellated forms found in the feces.

Patton himself (1910) performed certain experiments with H. muscae-domesticae in the host Musca nebulo

which convinced him that the flagellated forms as well as the cyst was infective. He found that if he fed "clean" flies of the species Musca nebulo upon spleen where infected flies had previously deposited their excreta, and that if he examined the intestinal contents of these clean flies within three hours from the time they were allowed to feed, flagellated herpetomonads would be present. On the second day he found numerous dividing forms. The infections produced in this manner were never so heavy as those found in "wild" flies.

Before I learned of Patton's work, I had carried out certain experiments with H. muscae-domesticae in the host Phormia regina, which seemed to demonstrate conclusively that the flagellated forms may transmit the infection under certain conditions. Because a different method of attacking the problem was employed and because my results amplify as well as confirm Patton's work, it seems well to publish them.

"Clean" (uninfected) flies were raised in the laboratory by the following method: A number of adult flies were put in a covered jar with a piece of raw flesh or ground beef. After the female flies had deposited their eggs upon the meat, it was transferred to another jar with an inch or two of sawdust on the bottom and covered with cloth. The

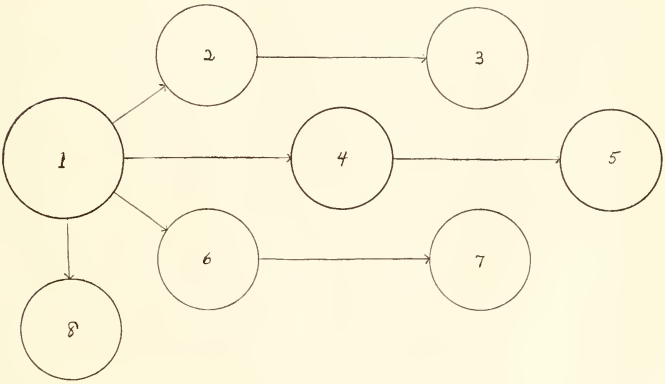
young larvae fed upon the meat until they reached the adult larval stage, when they migrated down into the sawdust and pupated. Very soon after hatching the flies were etherized or chloroformed and distributed among jars covered with cheese-cloth. The food of the fly consisted of only a slightly alkaline (Ph 8) dilute sugar solution. The sugar solution, jars, and cheese-cloth were boiled before using, and while in use were kept inside a bookcase to prevent infected flies from depositing their faeces upon the covers of the jars.

The "clean" flies were distributed so that one container held about thirty flies and seven others twelve flies each. They were fed sugar solution shortly after hatching, and then starved for twenty-four hours. At the end of that time a number of wild flies were examined microscopically for parasites, and the anterior ends of several heavily infected intestines were teased up in 0.4% NaCl solution and fed to the flies in the jar containing thirty flies, which we shall designate as jar 1. Most of the flies fed upon the infected material, and within a few minutes all the liquid had either been eaten by the flies or had dried up.

At the end of intervals of one, twelve, twenty-four, and seventy-two hours from the time of feeding the in-

Text, Fig. 2

A diagram representing the plan used in the fly infection experiment. The circles represent the glass jars containing flies; the lines, the time intervals between the time when the contents of the intestines of infected flies from containers, to the left of the line, were fed to the flies in the containers to the right; e. g., one hour after the flies in jar 1 were fed infective material a few flies were removed from that jar and examined microscopically for flagellates. The material in which active flagellates were found was at once fed to the flies, in jar 2.



fected liquid, flies were removed from jar 1 and examined for flagellated forms. Upon each examination flies were found which still held in their intestines some of the flagellates which they had ingested at the time of feeding the infected material. The intestinal contents of such flies was at once fed to clean flies in other jars which we shall designate as jars 2, 4, 6, and 8 (see text fig. 2).

Five hours after feeding, several of the flies in jar 2 were examined, and the flagellate stage was found to be present in the intestines of some of them. Immediately the infected intestinal contents was fed to flies in jar 3. In like manner some of the flies in jars 4 and 6 were examined for flagellates at respective intervals of 12 and 24 hours from the time of the infective feeding, and the intestinal contents from each was fed to the clean flies in another container (jars 5 and 7). Further, flies were removed from jar 1 at periods of 96, 120, 144, and 168 hours, and 15 days after the original infective feeding.

The examination of flies from jar 1 at intervals of 1, 12, 24, 72, 96, 120, 144, 168 hours, and 15 days from the time of attempted infection proved that the ingested flagellates and the flagellates which arise from them by binary fission may be found in the intestine after these

periods of time. At intervals of ten days after the time they fed upon the infected material, the flies in jars 2, 3, 4, 5, 6, 7, and 8 were examined microscopically for parasites. From one-third to three-fourths of the flies which remained alive in each jar carried infections, most of them fairly heavy. The tremendous increase in numbers showed that the flagellated forms originally ingested by the flies had succeeded in establishing themselves and multiplying in the flies' intestines.

There was a two-fold reason for passing the parasites through two and three hosts. First, if there had by chance been any cysts in the original infective material which was taken from the anterior ends of the alimentary tracts of infected "wild" flies, the various time intervals would have given them time either to excyst or to be carried passively through the flies' intestines. Second, it proved that there is no definite physiological cycle which must be undergone before a Herpetomonas ingested by one host is infective to another. It will be remembered that Minchin and Thompson (1915) found that within one-half hour after a rat flea has ingested the blood of a rat infected with Trypanosoma lewisi, a physiological change occurs which makes the trypanosome incapable of infecting another rat when injected into it, until

after a developmental process requiring at least five days has taken place. These fly experiments indicate that there is no comparable obligatory cycle in H. muscae-domesticae which must take place before one Phormia regina fly can be infected from another.

Proper controls of non-infected flies were examined throughout the experiment. Ten flies hatched at the same time as those used in the experiments were examined for parasites at the beginning of the experiment and were found to be negative. Fifteen others were kept in a separate container and ten days after hatching were found to be negative.

Fly experiments of this nature would be of little value if the infection could be transmitted "hereditarily" through the ovum. Prowazek (1904) claims to have demonstrated that the ova of Sarcophaga haemorrhoidalis Fall may be invaded by Herpetomonas, where they undergo a developmental process and infect some of the larvae. Since Prowazek's observation attempts to transmit the infection "hereditarily" have met with failure. Roubaud (1912) working with Pycnosoma putorium and Patton (1910) working with Musca nebulo were unable to demonstrate hereditary transmission of Herpetomonas. Patton states, "My experiments have clearly demonstrated that Herpetomonas muscae-domesticae is not transmitted through the eggs of Musca nebulo."

In my various experiments with various species of diptera, I have employed more than six hundred flies, largely bred from infected wild flies. A generous sampling of these flies, about 140, were examined at various intervals after hatching for control purposes. In no case have I ever found so much as one control fly infected. These flies belonged to the species mentioned in the first part of this paper. The two following experiments were done with Sarcophaga.

A female Sarcophaga bullata captured in August carried the heaviest infection with Herpetomonas muscae-domesticae which I have ever found in a fly. The abdomen carried a number of larvae which were placed on a piece of fresh meat and from them about twenty-five adult flies were raised. Ten of these flies were examined at once after hatching and fifteen others after an interval of five days. Herpetomonas was not found to be present in the intestines.

A female Sarcophaga securifera captured the same month was found to have a fairly heavy infection with Herpetomonas muscae-domesticae. Its brood was kept under the same conditions as that of S. bullata; of the twenty-one flies which hatched, none were infected after two days.

It must be admitted that neither my results nor those of other workers prove conclusively that hereditary in-

fection with Herpetomonas may not occur exceedingly rarely in muscoid flies. At any rate the evidence for it is very meagre, and it is impossible to accept this part of Prowazek's work until more positive experimental evidence is produced.

The exact status of larval infections is somewhat complicated. Prowazek (1904) found infected Sarcophaga larvae. Patton (1910) reported that the full grown larvae of Musca nebulo were not infected, but that he had found a few long flagellates in very young larvae which had fed upon the bodies of dead infected flies. At that time he reported that the full-grown larvae, pupae, and adults bred from such larvae were uninfected. More recently (1921) the same author reports finding larval infections, and that the infection is carried through the pupae stage to the adult. Mackinnon (1910) reported larval infections in the case of Scatophaga and Homolomyia, but was unable to find infected larvae of Musca domestica. Strickland (1911) was unable to find infected Lucilia larvae.

During the months of July and August, 1922, I examined two hundred fly larvae, principally Lucilia and Phormia, in various stages of development, taken from the most exposed places in Westport dump (Baltimore). No infected larvae could be found. I also examined sixty Musca domestica larvae taken from a pile of horse manure, but I found no infections. An

attempt was made to experimentally infect the larvae of Sarcophaga bullata, the adults of which are parasitized to the extent of about fifteen per cent in nature. A small amount of ground beef was thoroughly mixed with the infected intestines of about twenty-five Phormia flies. The flagellates could easily be found microscopically in the liquid from the beef. Some of this material was fed to ten uninfected adult flies (Sarcophaga bullata). Ten days later three of them were found to be infected. Thus the ground beef was proved to be effective. About fifty young larvae were placed upon this beef, and then left there for twenty-four hours. At the end of forty-eight hours an examination of the intestines of ten larvae gave negative results. Examinations of the larvae made each succeeding day for one week were also negative.

It is difficult to draw any conclusions from the conflicting observations of various authors, but it seems that one of two factors must be at work here. It is possible that the larvae of certain species of muscoid flies are capable of infection with Herpetomonas, and that certain others are not. Then there is the other possibility that certain environmental conditions, relating to temperature, moisture, character of food, flora of the larval intestine, etc. are factors which determine whether or not larvae can

be infected. Much more work needs to be done along this line in order to determine what are the factors which determine the presence or absence of infections in fly larvae.

Nomenclature. *

There has been much controversy concerning the generic names Leptomonas and Herpetomonas. Kent (1881) gave the former name to a flagellate from a free-living Nematode, Trilobus gracilis, and the latter to the flagellate from the house-fly, the descriptions being so similar that it is impossible to make any important distinction between the two. The fact that the first reviser, Butschli (1884) united the genera Herpetomonas and Leptomonas and selected the former as the name for the composite genus, and the further fact that the type species of Herpetomonas is easily obtainable, while the type species of Leptomonas is not, makes it obligatory that the name Herpetomonas be accepted and Leptomonas rejected. (See International Rules of Zoological Nomenclature, Article 28). This decision is necessary in spite of the fact that in Kent's original work Leptomonas has page priority over Herpetomonas.

* The main conclusions regarding nomenclature were reached after communication with Dr. C. W. Stiles, to whom I am very much indebted for his opinion on the subject.

The specific name *muscae-domesticae* was originally ascribed to Burnett by Stein (1878), and later ascribed to Minchin (1911), Wenyon (1912), and other writers subsequent to Stein. In spite of the fact that we have carefully read all the references to Burnett given by Stein, Kent, and Wenyon, as well as a number of other papers by Burnett, we are unable to find that he ever referred to this species other than as "Bodo of the common House-fly." The name *muscae-domesticae* has, therefore, to be referred to Stein, although previous to Stein's work Leidy had proposed the name *Bodo muscarum* for a "proto-elminth" from from the fly *Musca domestica*. In the absence of either a recognizable description of the parasite or a reference to Burnett's description we must consider *Bodo muscarum* a *nomen nudum*. The correct name of this protozoon is, therefore, *Herpetomonas muscae-domesticae* (Stein, 1878, nec Burnett) Kent 1881.

Chief synonyms of the parasite as described from *Musca domestica* are

Bodo of the common house-fly, Burnett, 1852.

Bodo muscarum Leidy, 1856, *nomen nudum*,
does not mention Burnett.

"*Bodo muscae domesticae* Burnett" of Stein, 1878.

Cercomonas muscae-domesticae (Stein, 1878)
Stein, 1878.

Cercomonas musca Leuckart, 1879.

Lectomonas muscae-domesticae, Wenyon, 1911.

Conclusions.

1. Herpetomonas muscae-domesticae was found to be entozoic in the North American muscoid flies, Musca domestica, Phormia regina, Lucilia sericata, Calliphora erythrocephala, Cochliomyia macellaria, and Sarcophaga bullata.

2. The seat of infection is throughout the length of the alimentary canal. Infections may differ very much in respect both to the number of parasites present and to the localization of a particular stage of the parasite in the intestine.

3. The flagellate in its life history exhibits the adult long flagellated form, the cyst, and the intermediate stages from the crithidial to the trypaniform type.

4. This flagellate shows no indications of possessing a cytostome, nor does it ingest solid food, such as bacteria.

5. The flagellum, which arises from the basal granule, consists of four parts, the fine intracellular portion or rhizoplast, the marginal granule, the heavy extracellular portion, and the cytoplasmic sheath covering the extracellular portion.

6. The parasomal body stains with Janus green after the typical mitochondrial fashion.

7. The nucleus is vesicular in structure and neither in the resting stage nor the dividing stage exhibits a centriole.

8. The only method of multiplication found was binary fission. The nucleus divides by pannitosis, during which the chromatin is resolved into a number of fragments or chromosomes, usually four in each of the newly-formed daughter cells.

9. Feeding experiments were performed which indicated that the flagellated form of the parasite is infective under certain conditions, and that there is no obligatory cycle which must be completed before the parasites of one host are infective to another.

10. No evidence for hereditary transmission of Herpetomonas muscae-domesticae was found.

11. Attempts to find infected larvae or to experimentally infect them were failures. Infected larvae of some species of flies, however, have often been reported by other workers.

Explanation of plate. Obj. 1-20, oc 2 Leitz, drawn with the aid of a camera lucida.

1, the Herpetomonas found in Sarcophaga securifera.

2, an adult uniflagellated H. muscae-domesticae.

3, a biflagellated individual with an accidental arrangement of cytoplasmic granules posteriorly from the paranasal body simulating a rhizostyle.

4-6, other forms with the double flagellum.

7, a division stage where there appears to be two and three chromosomes in the daughter nuclei.

8, four chromosomes distinctly visible in each daughter nucleus.

9, parabasal body in process of dividing. It suggests here that an achromatinic filament joins the daughter nuclei.

10,11, other stages in division of parabasal body.

12, an abnormal condition in which most often of division where both daughter nuclei remain in the same cell, although the cytoplasm of the parent cell has commenced to divide so as to form daughter cells.

13-14, the daughter flagellates have almost completed the division process, the nuclear chromatin still remaining fragmented."

15, a "scooter."

16, the parabasal body has commenced to migrate posteriorly. The proximity of the parabasal body to the nucleus makes this a crithidial stage.

17, the parabasal body has migrated further toward the posterior end, and the cell has shortened noticeably. Very often in this stage the parabasal body is posterior to the nucleus.

18, an extremely small trypaniform type which is about to lose its flagellum to become a cyst.

19, a large cyst.

20, representative of the Grist flagellates stained by the Wright method. Parabasal body stains deep magenta; nucleus, pink.

21-33, drawings of the stages in nuclear division, not according to scale.

21-25, resting nuclei.

26-29, elongation of nucleus, first indication of division in the nucleus.

30, a nucleus drawn to an angle with the longitudinal axis. Very often the chromatin at this stage is fragmented.

31-33, nuclear appearances just prior to the constriction of the nucleus into two daughter nuclei. In Fig. 33, the nuclear chromatin has resolved itself into eight discrete granules, four of which will probably go with each daughter cell.

Bibliography

Alexeieff, A. 1912 - Notes sur les Herpetomonadidae. Arch. Zool. Exp. (5) 9 (Notes et Revue No. 2): XXIX-XXXVIII.

Alexeieff, A. 1913 - Introduction a la revision de la famille Herpetomonadidae. Arc. f. Prot. 29: 313-341.

Becker, E. R. 1923 - Observations on the morphology and life cycle of Crithidia gerridis Patton in the water-strider, Gerridis remegis Say. In press.

Brug, S. L. 1914 - Herpetomonas homalogyiae n. sp. Arc. f. Prot. 35: 119-126.

Hindle, Edward (1912) - What is the genus Leptomonas Kent? Parasit. 5: 128-134.

Kofoid, C. A. 1916 - The biological and medical significance of the intestinal flagellates. Proc. Soc. Pan. Amer. Sci. Congress, 1915-1916.

Mackinnon, D. L. 1910 - Herpetomonas from the alimentary tract of certain dung-flies. Parasit. 3:255-274.

Minchin, E. A. and Thomson, J. D. 1915 - The rat-trypanosome, Trypanosoma lewisi, and its relation to the rat-flea, Ceratophyllus fasciatus. Quart. Jour. Micro. Sci. 60 (N.S.): 463-692.

Patton, W. S. 1910 - Experimental Infection of the Madras bazaar fly, Musca nebulo Fabr. with Herpetomonas muscae-domesticae Burnett. Bull. Soc. Path. Exot. 3: 264-273.

Patton, W. S. 1921 - Studies on the flagellates of the genera Herpetomonas, Crithidia, and Rhynchoidomonas. No. 7, Ind. Jour. Med. Res. 9: 230-239.

Prowazek, S. v., 1904 - Die Entwicklung von Herpetomonas einem mit den Trypanosomen verwandten Flagellaten. Arb. a. d. Kaiserl. Gesundh. 20: 440-452.

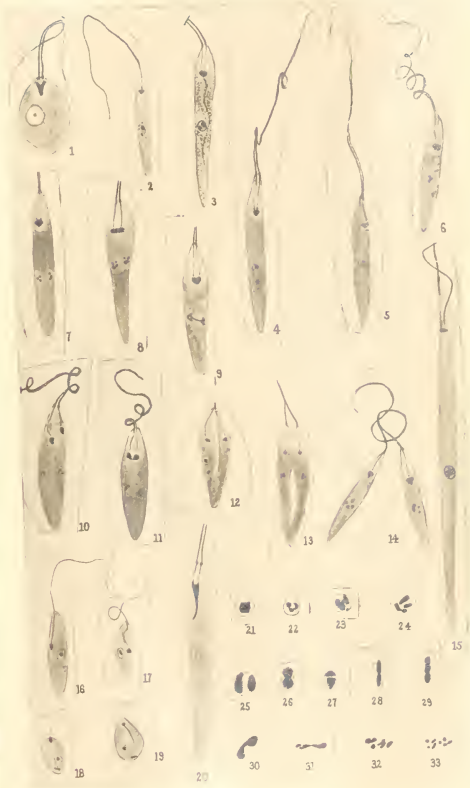
Roubaud, E. 1912 - Experiences de transmission de flagellés divers chez les Muscidés africains du genre Pyrophasma. C. R. Soc. Biol. 72: 502-509.

Strickland, C. (1911) - Description of a Herpetomonas parasitic in the alimentary tract of the common green-bottle fly, Lucilia sp. Parasit. 4: 222-236.

Swellengrebel, N. H. 1911 - Note on the morphology of Herpetomonas and Crithidia, with some remarks on "Physiological degeneration." Parasit. 4: 108-130.

Wenyon, C. M. 1913 - Observations on Herpetomonas muscae-domesticae and some allied flagellates. Arc. f. Prot., 30: 1-36.

Whitmore, F. R. 1911 - Prowazekia asiatica. Arc. f. Prot. 22: 370-378.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Transmission Experiments on the Specificity of Herpetomonas muscae-domesticae in Muscoid Flies. *

The large number of protozoan parasites which have been given specific names that refer to their animal hosts indicates the strong belief of parasitologists in host specificity. In many instances the results of morphological and experimental investigation have justified such a belief. The recent careful morphological studies of Simon (1922) and Hegner (1922), as well as the earlier studies of Kofoid and his co-workers, show that the species of Giardia living in man, meadow mice, rats, dogs, and rabbits are distinguishable. The feeding experiments of Simon (1922) suggest a rigid host specificity for Giardia. Failure to find animal reservoirs for human infections with Giardia, Endamoeba histolytica, and the three forms of human malaria have somewhat intensified the general belief in absolute host specificity.

The workers who have investigated the flagellate parasites of the non-biting muscoid flies have, as Alexeieff

* This paper is the third of a series concerning the haemoflagellates living in the intestines of insects. The writer is indebted to Drs. R. W. Hegner and W. H. Taliaferro for many helpful suggestions and criticisms. Thanks are due to Dr. F. M. Root for identifying the species of flies for me and for his friendly interest in the fly infection experiments.

(1913 a) pointed out, assumed that a Herpetomonas found in a host where it had not previously been noted is a new species. The result is that the literature contains descriptions of a large number of species of Herpetomonas which are hardly distinguishable. Alexeieff (1913 b) lists as synonyms of Herpetomonas muscae-domesticae, Leptomonas drosophilae Chatton and Alilaire, L. pycnosomae Roubaud, L. ampelophilae Chatton and A. Leger, and Herpetomonas luciliae Strickland. To his list I should add H. calliphorae Swingle, H. sarcophagae Prowazek, and H. homalomyiae Brug. In his various writings Patton has avoided the common error by noting that Herpetomonas muscae-domesticae may be present in a number of species of hosts.

Alexeieff (1913 b) justified his grouping of a large number of supposed species of Herpetomonas into three species on strictly morphological grounds. He argues that, since in the protozoa we can neither apply the specific test of cross fertilization nor use the delicate cultural reactions upon which bacteriologists depend, we must use morphological criteria alone in classifying protozoa. He further makes the debatable statement that physiological differences of specific importance between organisms have their recoil in the morphology.

It was for the purpose of determining how far Alexeieff's assumption of the identity of a number of the Herpetomonas entozoic in the common non-biting muscoid flies, based upon morphological grounds, would be substantiated by cross infection experiments, that these experiments were undertaken. To state the problem in a question, -- if the type of Herpetomonas known as Herpetomonas muscae-domesticae is morphologically alike in a number of different species of hosts, might there not be physiological differences which have not manifested themselves in the morphology of the flagellate markedly enough to be detectable, yet of sufficient importance to render the parasite incapable of living, multiplying, and establishing a normal infection in any species of host other than that in which it is found in nature?

An examination of a large number of "wild" flies captured during the summer of 1922 showed that flagellates of the type described by Prowazek (1904) as Herpetomonas muscae-domesticae may be present in a number of species of hosts. This flagellate may be found naturally in Phormia regina, Lucilia sericata, Musca domestica, Cochliomyia macellaria, Calliphora erythrocephala, and Sarcophaga bullata. Sixty-six per cent of Phormia regina were infected, but not over two or three per cent of Musca domestica, although a record of the

incidence of infection of the latter species was not kept.

The question may be raised as to whether the herpetomonads of these various species of flies are similar enough morphologically to be considered a single species. After studying a number of slides of the parasite from each species of host, it soon became evident that whether one or several species were represented, it was impossible to make any distinctions of specific importance. It is Herpetomonas muscae-domesticae which Prowazek (1904) incorrectly described as a biflagellate, because of its tendency to continue in a state of division, with which we are concerned here. It has the prominent marginal granules, basal granules, deep staining parabasal body, and vesicular nucleus with a thin achromatinic nuclear membrane. As was noted in a previous paper (Becker, 1923), two other types of Herpetomonas may be found in muscoid flies. The one is smaller than H. muscae-domesticae, does not exhibit the almost constant biflagellated appearance, and has no visible marginal granules. It is probably the same as "Crithidia" calliphorae Swellengrebel. The other type, like H. muscae-domesticae, shows a tendency toward the biflagellated division condition, has visible marginal and basal granules, but differs from H. muscae-domesticae in that it has a nucleus with a pronounced achromatinic nuclear membrane and a small central karyosome. But in regard to the

H. muscae-domesticae type, we must accept Alexeieff's opinion that this morphological type of flagellate occurs in a number of different species of hosts.

In order to determine whether or not the flagellates entozoic in the alimentary canal of a fly of one species may possess physiological characteristics which make it incapable of carrying on its vital activities in the alimentary canal of a host of a closely related species, and incapable of producing there a natural infection, certain factors are necessary.

First, naturally infected flies ("wild" flies) must be available.

Second, the flies to be tested for their susceptibility to parasites taken from hosts of another species must be "clean"; i.e., it must be definitely known that these flies are not already infected at the beginning of the experiment and that they are not exposed to accidental contamination after the time of intentional inoculation.

Third, the nature of the infection produced in the artificially infected flies must not be unlike that found in "wild" flies; i.e., the parasites inoculated per os into the new host must establish themselves in the alimentary canal, multiply, produce the same stages in their life history as in the host in which they were naturally found, and these parasites must be in-

fective to other "clean" flies under conditions simulating those in nature.

"Wild" infected flies of the species noted above were generally obtained by trapping, but in some cases the fly swatter was resorted to. "Clean" adult flies (except Musca domestica) were raised from the eggs in the laboratory by the method described in a previous paper (Becker, 1923). These flies belonged to the same species as the "wild" flies found to be infected with flagellates of the H. muscae-domesticae type; viz, Phormia regina, Lucilia sericata, Calliphora erythrocephala, Sarcophaga bullata, and Cochliomyia macellaria. Musca domestica was raised from larvae collected from horse manure. Sixty such larvae were examined for Herpetomonas and found to be negative. Adults examined immediately after hatching and one to two weeks after hatching were always found to be free from flagellates, as were the controls to be mentioned later. It is necessary to emphasize the fact that in this case the larvae and adults raised from the uninfected larvae were negative for Herpetomonas, because Patton (1921) recently discovered that the larvae of Musca nebulo were infected with a Herpetomonas which persisted through the pupa to the adult. The freedom of Musca domestica larvae from Herpetomonas infection in the vicinity of Baltimore may be explained by the low incidence of infection of the adults, not over three per

cent at most. As will be shown later, it is possible that even the adults got their infections from flies of other species. Immediately after hatching the adult flies were chloroformed and put into sterile glass jars covered with sterile cheese-cloth. For each species of "clean" fly there were seven containers, six of them to be used in the infection experiments and the seventh for a control.

The method of conducting the experiment was as follows. The flies were fed dilute cane sugar solution made slightly alkaline (Ph 8) until within twenty-four hours of the time when they were to be given an infected meal. After twenty-four hours of starvation the flies were given a meal consisting of a few drops of 0.4 saline solution in which had been emulsified part of the contents of an intestine heavily infected with Herpetomonas. After the time of inoculation per os the only food of the fly consisted of the dilute sugar solution. The alimentary canals of the inoculated flies were removed and examined microscopically for Herpetomonas from one to two weeks after the time of feeding the infected material. The following summary outlines briefly the important details and results of the experiments. The numbers of infected and uninfected flies are based upon the flies remaining alive, since many of the flies died and dried up during the interval between inoculation and examination.

I. Infection experiments with Musca domestica, hatched July 23rd.

A. 1. Fed Herpetomonas from Phormia regina July 25th.

Examined for parasites August 5th.

Negative 4.

Heavily infected 5. *

Lightly infected 4. *

2. Fed Herpetomonas from Lucilia sericata July 24th.

Examined for parasites August 5th.

Negative 1.

Heavily infected 7.

Lightly infected 3.

3. Fed Herpetomonas from Calliphora erythrocephala July 27th.

Examined for parasites August 5th.

Negative 4.

Heavily infected 5.

Lightly infected 6.

4. Fed Herpetomonas from Cochliomyia macellaria July 27th.

Examined for parasites August 5th.

Negative 0.

Heavily infected 10.

Lightly infected 1.

* Heavily infected means that the alimentary canal of the fly was literally filled with the flagellates. Lightly infected may mean anything from the condition where the flagellates are somewhat less in numbers to the condition where very few are present.

5. Fed Herpetomonas from Sarcophaga bullata

July 26th.

Examined for parasites August 5th.

Negative 2.

Heavily infected 2.

Lightly infected 5.

B. Controls - Examined for parasites August 5th.

Negative 16.

Positive 0.

II. Infection experiments with Phormia regina, hatched August 1st.

A. 1. Fed Herpetomonas from Musca domestica August 2nd.

Examined for parasites August 11th.

Negative 1.

Heavily infected 6.

Lightly infected 2.

2. Fed Herpetomonas from Lucilia sericata August 2nd.

Examined for parasites August 11th.

Negative 4.

Heavily infected 6.

Lightly infected 7.

3. Fed Herpetomonas from Calliphora erythrocephala August 2nd.

Examined for parasites August 11th.

Negative 4.

Heavily infected 7.

Lightly infected 4.

4. Fed Herpetomonas from Cochliomyia macellaria

August 2nd.

Examined for parasites August 11th.

Negative 4.

Heavily infected 6.

Lightly infected 4.

5. Fed Herpetomonas from Sarcophaga bullata August 2nd.

Examined for parasites August 11th.

Negative 2.

Heavily infected 8.

Lightly infected 4.

B. Controls - Examined for parasites August 11th.

Negative 17.

Positive 0.

III. Infection experiments with Lucilia sericata, hatched July 26th.

A. 1. Fed Herpetomonas from Phormia regina July 27th.

Examined for parasites August 5th.

Negative 0.

Heavily infected 15.

Lightly infected 1.

2. Fed Herpetomonas from Musca domestica July 28th.

Examined for parasites August 5th.

Negative 2.

Heavily infected 7.

Lightly infected 3.

3. Fed Herpetomonas from Calliphora erythrocephala

July 27th.

Examined for parasites August 5th.

Negative 2.

Heavily infected 6.

Lightly infected 7.

4. Fed Herpetomonas from Cochliomyia macellaria

July 27th.

Examined for parasites August 5th.

Negative 0.

Heavily infected 3.

Lightly infected 2.

5. Fed Herpetomonas from Sarcophaga bullata July 27th.

Examined for parasites August 5th.

Negative 3.

Lightly infected 4.

B. Controls - Examined for parasites August 5th.

Negative 23.

Positive 0.

IV. Infection experiments with Calliphora erythrocephala, hatched August 3rd.

A. 1. Fed Herpetomonas from Lucilia sericata August 7th.

Examined for parasites August 17th.

Negative 4.

Heavily infected 1.

2. Fed Herpetomonas from Phormia regina August 7th.

Examined for parasites August 17th.

Negative 4.

Heavily infected 1.

Lightly infected 2.

3. Fed Herpetomonas from Musca domestica August 7th.

Examined for parasites August 17th.

Negative 5.

Lightly infected 2.

4. Fed Herpetomonas from Cochliomyia macellaria

August 7th.

Examined for parasites August 17th.

Negative 5.

Lightly infected 1.

5. Fed Herpetomonas from Sarcophaga bullata August 7th.

Examined for parasites August 17th.

Negative 4.

Heavily infected 3.

Lightly infected 1.

B. Controls - Examined for parasites August 17th.

Negative 14.

Positive 0.

V. Infection experiments with Cochliomyia macellaria, hatched August 1st.

A. 1. Fed Herpetomonas from Calliphora erythrocephala August 2nd.

Examined for parasites August 11th.

Negative 4.

Lightly infected 3.

2. Fed Herpetomonas from Lucilia sericata August 2nd.

Examined for parasites August 11th.

Negative 7.

Lightly infected 3.

3. Fed Herpetomonas from Phormia regina August 2nd.

Examined for parasites August 11th.

Negative 6.

Lightly infected 2.

4. Fed Herpetomonas from Musca domestica August 2nd.

Examined for parasites August 11th.

Negative 4.

Heavily infected 4.

Lightly infected 4.

5. Fed Herpetomonas from Sarcophaga bullata August 2nd.

Examined for parasites August 11th.

Negative 4.

Heavily infected 2.

Lightly infected 2.

B. Controls - Examined for parasites August 11th.

Negative 10.

Positive 0.

VI. Infection experiments with Sarcophaga bullata, hatched August 8th.

A. 1. Fed Herpetomonas from Cochliomyia macellaria

August 9th.

Examined for parasites August 21st.

Negative 4.

Heavily infected 2.

Lightly infected 2.

2. Fed Herpetomonas from Calliphora erythrocephala

August 9th.

Examined for parasites August 21st.

Negative 5.

Heavily infected 2.

Lightly infected 4.

3. Fed Herpetomonas from Lucilia sericata August 9th.

Examined for parasites August 21st.

Negative 4.

Heavily infected 4.

Lightly infected 6.

4. Fed Herpetomonas from Phormia regina August 9th.

Examined for parasites August 21st.

Negative 3.

Heavily infected 3.

Lightly infected 1.

5. Fed Herpetomonas from Musca domestica August 9th.

Examined for parasites August 21st.

Negative 4.

Heavily infected 2.

Lightly infected 1.

B. Controls - Examined for parasites August 11th.

Negative 12.

Positive 0.

In addition to these experiments a number of laboratory bred flies belonging to the species Morellia micans and Sarcophaga securifera were fed Herpetomonas from Lucilia sericata. An examination made nine days later showed that out of nine of the former, one was infected; and out of fourteen of the latter, two were infected. Unfortunately no controls were kept.

It might be well also to mention here that attempts to infect Phormia, Lucilia, and Musca flies with Herpetomonas from Drosophila melanogaster were unsuccessful. Likewise, attempts to infect Drosophila melanogaster with Herpetomonas from Phormia regina were unsuccessful. The experiment needs to be repeated.

The results of these experiments indicate very plainly that the Herpetomonas which occurs naturally in the six species of muscoid flies mentioned above is non-specific for its host to the extent that parasites from any one of the

hosts can produce an infection in the other five. In many of the infections noted as heavy, the flagellates were as numerous as in the heaviest infections found in nature, in which the intestine is literally blocked with its inhabitants. So far as could be ascertained, the same stages in the life history were found in the inoculated flies as are found in naturally infected "wild" flies; viz, the adult flagellate, the trypaniform, the cyst, and dividing stages.

After some of these cross-infection experiments had been completed, it occurred to the writer that in nature a fly of one species must often ingest by fecal contamination of its food flagellates indigenous to other species. Perhaps some of these accidentally ingested organisms had originally come from the same species of fly as the one to which the infected material was fed. In such a case it might follow that the infection which appeared in the inoculated fly might actually have been caused by one or more parasites, which originally came from the same species of fly as the intentionally inoculated fly, and that the infection so produced did not come at all from the parasites indigenous to the other species. For example, a fly of the species Musca domestica might eat a drop of liquid garbage upon which a fly of the species Phormia regina had previously deposited its excreta contaminated with cyst and flagellated forms of Herpetomonas. If now the contents of the intestine

of this Musca domestica were fed to a "clean" Phormia regina, it is quite certain that the Phormia would become infected whether or not the parasites supposedly indigenous to Musca domestica played any part in producing the infection.

It was decided to pass the Herpetomonas from one species of fly through a number of hosts of different species in order to eliminate this possibility of error. Accordingly, the following two experiments were carried out.

Experiment A.

August 15. Herpetomonas from "wild" Lucilia sericata was fed to "clean" Phormia regina.

August 30. The intestinal contents of one of the heavily infected Phormia regina (inoculated August 15th) was fed to "clean" Sarcophaga bullata.

September 7. The intestinal contents of one of the heavily infected Sarcophaga bullata (inoculated August 30th) was fed to "clean" Lucilia sericata.

September 20. Examination showed that some of the Lucilia sericata (inoculated September 7th) were infected.

Experiment B.

September 24. Herpetomonas from "wild" Lucilia sericata was fed to "clean" Sarcophaga bullata.

October 7. Examination showed that some of the Sarcophaga bullata flies inoculated September 24th were infected with Herpetomonas. Ten which were not examined were placed in a jar containing a number of "clean" Phormia regina flies.

October 31. Six of the seven Phormia regina remaining alive were found to be infected with Herpetomonas. Three of the Sarcophaga bullata which remained alive were infected with Herpetomonas.

From ten to twenty of each lot of laboratory bred flies were examined as a precaution against accidental contamination and "hereditary" transmission through the ovary. No laboratory bred flies were ever found to be infected which had not been purposely inoculated per os.

The results of these experiments preclude the possibility that the infections were carried out with contaminated material. It is unthinkable that the parasites from Lucilia would passively be carried through Phormia and Sarcophaga, and twenty-three days after the time they were taken from Lucilia again be infective to it (Experiment A). The further likelihood that the first Lucilia carried in its intestine flagellates indigenous to Phormia and Sarcophaga is slight.

Experiment B justifies much the same conclusions as Experiment A, and in addition shows that herpetomonads which were taken from one species of fly and passed through two hosts of another species could infect a fourth species by fecal contamination, the probable natural method of transmission of the parasite. It might be added that these two experiments demonstrate the non-specificity of H. muscae-domesticae quite as well as the previously noted experiments, although not so many species of flies were used.

It was found that one species of fly might be infected with herpetomonads from other species by inoculation per os with the contents of the entire infected intestine diluted with saline solution. The question arises whether cross-infection of species occurs in nature, or whether in nature certain peculiar biological conditions obtain which prevent this, as Roubaud (1912) has suggested.

If a piece of putrifying meat is put outside the window on a warm summer day and watched, one can observe that many different kinds of flies alight upon the meat to feed or to deposit their eggs. I have observed Phormia and Lucilia in large numbers, Sarcophaga of various species, Calliphora, Cochliomyia, Musca domestica, Muscina stabulans, and other species of flies settle upon the same piece of meat in the course of one-half of an hour. Apparently, conditions are quite favor-

able for contamination of the food of a fly with excreta from flies of other species.

In order to determine whether or not natural cross-infection actually obtains, the following experiment was carried out.

Experiment C. About fifty "wild" Lucilia were captured and released in a glass aquarium jar in the bottom of which was a dead rat with intestines exposed. The flies were given an opportunity to contaminate the rat with their feces for a period of two hours. Fifteen of the flies were examined for Herpetomonas, nine of them being positive.

These Lucilia flies were released, and a number of Phormia, Lucilia and Sarcophaga flies were liberated within the aquarium jar. After they had fed upon the rat for six hours, the flies were etherized, placed in clean glass containers, and fed dilute sugar solution. Twelve days later the flies were examined for Herpetomonas with the following results:

Species	Number examined	Positive infections
Lucilia sericata	18	10
Phormia regina	21	15
Sarcophaga bullata	10	2
Controls		
Lucilia sericata	10	0
Phormia regina	10	0
Sarcophaga bullata	10	0

It is evident from the above data that the infected Lucilia flies could transmit the infection to flies of the same and of two other species under conditions as nearly natural as is possible to make them in the laboratory.

Discussion

Roubaud (1912) attempted to infected Pycnosoma putorium with "Corcoplasma" mirabilis, Leptomonas from Pyrellia, and Cystotrypanosoma gravi from Glossina. In the absence of positive results he drew the conclusion that the facts indicate a great degree of specificity of the different types of flagellates for their muscoid hosts. Likewise, the morphological studies of Chatton and Leger (1911) seem to indicate that each species of Drosophila has its own type of parasite. These results are hardly what one should expect, judging not only from the experiments with Herpetomonas muscae-domesticae, but also from the evidence furnished in the case of trypanosomes and their intermediate hosts. It has been demonstrated that Trypanosoma lewisi develops in almost every species of flea with which experiments have been carried out. Furthermore, it develops in the rat louse, Haematopinus spinulosus, and in the bed-bug, Cimex lectularius (Minchin and Thompson 1915). Trypanosoma cruzi develops in four species of the genus Triatoma and in one species of Rhodnius. It is not surprising that Herpetomonas, which is closely related to Trypanosoma biologically, should seem

a lack of rigid adaptation to a particular species or genus of host.

Alexeieff (1913) laid down the two following principles as a basis for general parasitology:

1. One species of parasite may be harbored by different hosts, sometimes quite removed from each other in the zoological scheme.

2. A host may harbor two or more closely related species of parasites.

There can be no disputing the correctness of these two fundamental contents. The herpetomonads of at least six muscoid flies are easily transferrable from one species of host to another. Then there is the evidence from the non-specificity of trypanosomes for their intermediate hosts. This and much more evidence might be brought to bear in support of the first principle. Probably as many facts could be marshalled for the second principle as for the first. The muscoid fly Calliphora erythrocephala is known to harbor two distinct types of Herpetomonas (Swellengrebel 1911). The rat flea may harbor both Herpetomonas and T. lewisi often to the confusion of the investigator. Anyone without practical experience who attempts to distinguish between the free-living stages of the human amebae, Endamoeba coli and Endamoeba histolytica will realize the truth of this second principle of Alexeieff.

While it is important to realize the non-existence of absolute specificity of the parasites for their hosts, it must be kept in mind that some parasites do manifest a most rigid choice of host. The human plasmodias, trypanosomes of some of the lower animals, and many of the intestinal protozoa of man have never been successfully cultivated in any animal other than their indigenous hosts. But instances of this nature should not supply an a priori justification for neglecting to investigate the specific host relationships of animal parasites.

SUMMARY

1. The type of Herpetomonas known as H. muscae-domesticae was found to be entozoic in the alimentary canals of the muscoid flies Musca domestica, Chrysomia regina, Lucilia sericata, Sarcophaga bullata, Cochliomyia macellaria and Calliphora erythrocephala.

2. The flagellate from any one of these six species of "wild" naturally infected flies was capable of producing a natural infection in the other five species of "clean" laboratory bred flies when inoculated per os (Experiments 1-6).

3. That cross-infection was not due to accidental contamination was demonstrated by passing the parasites through a number of hosts of different species (Experiments 2 and 3).

4. Such infected flies were "carriers" capable of infecting other flies by fecal contamination of the food or proboscis (Experiment B).

5. Cross-infection of species by food contamination can and probably does occur in nature, as was proven by an experiment in which natural conditions were simulated (Experiment C).

6. It is extremely probable that Herpetomonas muscae-domesticae, H. luciliae, H. calliphorae, H. sarcophagae, and the Herpetomonas from Chermia and Coelliomyia flies all represent one and the same species.

Literature Cited

Alexeieff, A.

1913 a - Quelques remarques a propos de la specificite parasitaire. Zool. Anz. 41: 17-20.

Alexeieff, A.

1913 b - Introduction a la revision de la famille Herpetomonadidae. Arch. f. Prot. 29: 313-341.

Becker, E. R.

1923 - Observations on the Morphology and Life History of Herpetomonas muscae-domesticae in North American muscoid flies. In press.

Chatton and Lwoff.

1911 - Documents en faveur de la pluralite des especes chez les Lentomonas des Drosophiles. Remarques sur leur morphologie, C. R. Soc. Biol. 71: 383-385.

Hegner, R. W.

1922.- A comparative study of the Giardias living in man, rabbit, and dog. Am. Jour. Hyg., 2: 442-454.

Minchin, E. A., and Thomson, J. G.

1915 - The rat trypanosome, Trypanosoma lewisi, and its relation to the rat flea, Ceratophyllus fasciatus. Quart. Jour. Micr. Sci. 60 (N. S.): 463-692.

Patton, W. S.

1921 - Studies on the flagellates of the genera Herpetomonas, Crithidia, and Rhynchoidomonas. No. 7. Ind. Jour. Med. Res. 9: 230-239.

Prowazek, S.

1904 - Die Entwicklung von Herpetomonas, einem mit den trypanosomen verwandten flagellaten. Arb. a. d. Kaiserl. Gesundh. 20: 440-452.

Raubaud, E.

1912 - Experiences de transmission de flagelles divers chez les muscides africans du genre Pycnosoma. C. R. Soc. Biol. 72: 508-509.

Simon, C. E.

1922 - A critique of the supposed rodent origin of human giardiasis. Amer. Jour. Hyg. 2: 406-434.

Swellengrebel, N. H.

1911 - Note on the morphology of Herpetomonas and Crithidia with some remarks on physiological degeneration. Parasit. 4: 108-130.

STUDIES ON THE RELATIONSHIP BETWEEN INSECT FLAGELLATES AND LEISHMANIA.

The intimate biological relationships which are known to exist between protozoan parasites of man, belonging to the genera Leishmania and Trypanosoma, and those of insects, belonging to the genera Crithidia and Herpetomonas, have led to extensive investigations of the flagellates of insects. Since Rogers' discovery that the leishmania body of kala azar flagellated in citrated human blood and became morphologically a Herpetomonas, it was thought that the Herpetomonas and Crithidia of insects might hold the key to the biological character and mode of transmission of human leishmaniosis. It is suspected that kala azar is an insect carried disease, but positive evidence for this is lacking. The experiments of Rogers and Price, working in Assam, demonstrated, however, that a distance of a few hundred yards between an infected and an uninfected camp is sufficient prophylaxis to the uninfected camp. This suggests that, if the disease is insect borne, the transmitter is not a winged insect.

Many of the biological aspects of the problem seemed to have been solved by the animal inoculation experiments of Laveran and Franchini, which have led many to believe that the human host was but the blind alley of a natural parasite. In general, their experiments consisted of the production of artificial leishmaniosis in laboratory animals, principally white mice and white rats, by intraperitoneal inoculations and feeding the herpetomonads and crithidias found naturally, so far as is known, only in the alimentary canals of insects. Among the flagellates employed were Herpetomonas ctenopsyllae, H. ctenocephali, H. jaculum, H. pattoni, H. culicis, H. muscae-domesticae, H. sarcophagae, H. phlebotomi, Crithidia melophagia and C. fasiculata. More recently Franchini (1922) has reported successful inoculation of white mice with protozoa found in the latex of various plants belonging to the Euphorbiaceae.

The infections produced in the rats and mice varied somewhat in their character, but certain things can be said of them in general. From three days to a week after the time of inoculation it was possible to find free or intracellular parasites, usually of the leishmania type, in the peripheral blood. Frequently flagellated

forms and Anaplasma bodies in red cells were encountered. Some of the infections resulted in severe anemia and in death of the host. If the animal died either as a result of the infection or was sacrificed, the spleen and sometimes the liver were enlarged. Tissue smears of the spleen, liver, and bone marrow often revealed the presence of the leishmania and flagellated forms of the parasite. In some cases it was possible to transmit the infection from one animal to another by subinoculation of infected blood. Attempts to cultivate the parasites from the blood and tissues of the parasitized animals were successful in only a few cases.

Fantham and Porter (1916) published a series of experiments on the inoculation of various mammals, birds, amphibians, and fish with insect Herpetomonas and Crithidia. From their reports it would seem that they were most strikingly successful in their efforts to produce flagellosis in animals of all these classes.

Hoare (1921) carried out a series of experimental animal inoculations with results quite contrary to those of Laveran and his collaborators, and of Fantham and Porter. Crithidia melophagia, Herpetomonas callipharae and H.jaculum

were inoculated into mice with negative results. Sticklebacks, newts, and frogs did not become infected when inoculated with H. jaculum. The results justified the conclusion that "artificial infection with insect flagellates requires further study and the results already recorded do not permit as yet of forming general conclusions as to the pathogenicity of insect flagellates and their relation to Leishmania."

Glaser (1922) recently reported his unsuccessful attempts to produce herpetomoniasis in four white and two wild mice, one rat, and one guinea pig, by intraperitoneal inoculation with H. muscae-domesticae.

In view of the directly opposed results obtained by these prominent investigators, the writer undertook some similar experiments with the hope of obtaining more evidence in regard to the possibility of producing artificial leishmaniosis in laboratory animals by means of insect flagellates. If the results had been positive, the problem would have been much nearer a solution. But fortunately or unfortunately the results were negative, and can have such value only as is ordinarily attributable to negative results.

A. Experiments with half-grown white rats. Animals were in good health when killed unless otherwise stated.

1. Rats inoculated intraperitoneally with Crithidia gerridis.

(a) Inoculated April 28. Blood smears negative May 4, 7, 14, and 24. On May 31 rat became sick and refused to eat. Blood smears negative, anemia marked. Rat killed June 1. Smears of liver, spleen, and bone marrow negative. Cultures on NNN medium from blood and spleen gave heavy bacterial growth.

(b) Inoculated April 28. Blood smears negative May 4, 7, 14, 24, and 31. Rat killed July 1. Smears from liver, spleen, and bone marrow negative. Cultures on NNN medium negative after 21 days.

(c) Same as (b).

(d) Same as (b).

(e) Inoculated April 28. Died of acute peritonitis the next day.

2. Rats inoculated intraperitoneally with Herpetomonas muscae-domesticae.

(a) Inoculated May 11. Died of acute peritonitis the next day.

(b) Same as (a).

(c) Inoculated May 11. May 12, the rat sickened. Blood smears negative for parasites, anemia marked. Anaplasma-like bodies in some red cells. May 15, rat appears normal again, blood negative for parasites. Blood smears negative May 31 and June 15. Rat killed July 1 while in apparently good health. Cultures of heart's blood and spleen on NNN medium negative after 21 days.

(d) Inoculated May 12. Blood smears negative except for anaplasma-like bodies in red cells, May 15. Blood smears negative May 31 and June 15. Rat was killed July 1. Smears from liver, spleen, bone marrow, and heart's blood negative for parasites. Cultures on NNN medium negative after 21 days.

(e) Same as (d) except that no anaplasma-like bodies were ever found.

(f) Inoculated May 24. Blood smears negative

May 27, June 8, and June 15. Rat was killed July 1. Smears of liver, spleen, and bone marrow negative for parasites. Cultures negative.

3. Rats inoculated intraperitoneally with Trypanosoma melaphagium Flu from the sheep-tick (formerly known as Crithidia melophagia.) Each animal was injected with the intestinal contents of two heavily infected sheep-ticks.

   (a) Inoculated June 28. Stained smear and fresh blood negative for parasites July 2, 7, 13, 17. The rat died of an unknown cause July 28. Blood, spleen, liver, and bone marrow negative for parasites.

   (b) Inoculated June 28. Stained smears and fresh blood negative for parasites July 2, 7, 13, 17, 28. Sacrificed Aug. 7. Blood, liver, spleen, and bone marrow negative. Cultures on NNN medium from blood, spleen, liver, and bone marrow negative.

   (c) Same as (b).

   (d) Inoculated June 28. Stained smears and fresh blood negative July 2, 7, 13, 17, and 28. Died of unknown cause, Aug. 3.

Examination of heart's blood, spleen, liver, and bone marrow negative for parasites.

(e) Inoculated June 28. Stained smears and fresh blood negative July 2, 7, 13, 17, and 28. On July 28 blood platelets, some of which resembled leishmania bodies, were especially numerous. The rat was sacrificed, and cultures on NNN medium were made from the heart's blood, liver, spleen, and bone marrow. They were negative after three weeks. Leishmania bodies could not be found in sections of the liver and spleen.

B. Experiments with white mice.

1. Mice inoculated intraperitoneally with Trypanosoma melophagium from sheep-tick.

(a) Inoculated June 28. Examination of fresh blood and stained dried smears negative for parasites July 2, 7, 13, 17, and 28. Aug. 3 the mouse was found dead and partially devoured by the other mice.

(b) Inoculated June 28. Examination of fresh blood and stained dried smears negative for parasites July 2, 7, 13, 17, and 28. Sacrificed

Aug. 7. Smears of heart's blood, spleen, and liver negative for parasites. Cultures on NNN medium from blood and spleen negative after 3 weeks.

(c) Same as (b).

C. Experiments with other animals.

1. Rabbit inoculated intraperitoneally with Trypanosoma melophagium from sheep-tick. Inoculated June 28. Examination of fresh blood and smears negative July 2, 7, 13, 17, 28, and Aug. 3. The rabbit was not sacrificed.

2. Guinea pig inoculated intraperitoneally with Trypanosoma melophagium from sheep-tick. Inoculated June 28. Examination of fresh bloodand smears negative, July 2, 7, 13, 17, 28 and Aug. 3. The animal was not sacrificed.

The results of these experiments were negative in so far as the production of flagellosis in vertebrates with flagellates from insects' guts is concerned. It is interesting to note that the normal blood platelets of rats and mice often bear a marked resemblance to the leishmania bodies, and the deception may be greater when a blood platelet is found lying upon a red cell. The occasional presence of anemia and ana-

plasma-like bodies could in no way be attributed to the presence of flagellates. It is possible that the anaplasma-like bodies are comparable to Howell-Jolly bodies.

Recent work indicates that Crithidia melophagia is in reality a stage in the life history of the sheep trypanosome. What we should expect to find in the blood of inoculated experimental animals is, then, trypanosomes, rather than the Leishmania bodies which Laveran and Franchini found. Careful examination of the fresh blood and stained dried smears, however, of animals inoculated with this flagellate disclosed the presence of neither trypanosomes nor other flagellates. If this is indeed a trypanosome, the results of these experiments indicate its great specificity for the sheep.

Conclusions and discussion.

Attempts to infect laboratory animals with Crithidia gerridis from the water-strider, Herpetomonas muscae-domesticae from muscoid flies, and Trypanosoma melophagium from the sheep-tick were unsuccessful. In the experiment seventeen white rats, three white mice, a rabbit, and a guinea pig were employed.

The failure of other workers and the writer to duplicate the results of Laveran and his collaborators, and of Fantham and Porter, does certainly show that artificial leish-

maniosis is much more difficult to realize in the laboratory than their publications indicate. Whether the difficulty of repeating their work lies in the strains of the laboratory animals, the insect parasites, or the technique, it is at present impossible to say.

Patton accepts the animal inoculation work to the extent that he would use the term herpetomoniasis rather than leishmaniasis. The following statement of his, however, is quite significant: "Transmission experiments on white mice would give us some valuable information as to whether the species of insect flagellates found in India are capable of living in the tissues of smaller laboratory animals. I would, however, warn the observer who undertakes such experiments that the white Japanese performing mice, which are usually sold in India, seem to be of little use for such experiments. I have inoculated many of these mice with several species of Herpetomonas, including H. donovani, but have never yet succeeded in obtaining any positive results."

Literature Cited.

1. Fantham, H. B. and Porter, Annie.

1916. The significance of certain natural flagellates of insects in the evolution of disease in vertebrates. Jour. Parasit. 2: 149-166.

2. Franchini, F.

1922. Essais d'inoculation aux souris blanches du latex parasite de differentes especes d'euphorbes. Ann. Inst. Past. 36: 873-881.

3. Franchini, F. and Mantovani, M.

1915. Infection experimentale du rat et la souris par Herpetomonas muscae-domesticae. Bull. Soc. Path. Exot. 8: 109-111.

4. Glaser, R. W.

1922. Herpetomonas muscae-domesticae, its behavior and effect in laboratory animals. Jour. Parasit. 8: 99-108.

5. Laveran, A. and Franchini, G.

1913. Infections experimentales de la souris par Herpetomonas ctenocephali. C. R. Acad. Sci. 157: 423-426.

Literature Cited (Continued)

6. Laveran, A. and Franchini, G.

1913a. Infections experimentales de mammiferes par des flagelles du tube digestif de Ctenocephalus canis et d'Anopheles maculipennis. C. R. Acad. Sci. 157: 744-747.

7. Laveran, A. and Franchini, G.

1914. Infections de mammiferes par des flagelles d'invertebres. Bull. Soc. Path. Exot. 7: 605-612.

8. Laveran, A. and Franchini, G.

1914a. Infections naturelle du rat et de la souris au moyen de puces de rat parasitees par Herpetomonas pattoni. C. R. Acad. Sci. 158: 450-453.

9. Laveran, A. and Franchini, G.

1914b. Infections de la souris au moyen des flagelles de la puce du rat, par la voie digestive. C. R. acad. Sci. 158: 770-772.

10. Laveran, A. and Franchini, G.

1919. Infection des souris blanches a l'aide des cultures de Herpetomonas ctenocephali. Bull. Soc. Path. Exot. 12: 379-383.

Literature Cited (Continued)

11. Laveran, A. and Franchini, G.

1916b. Sur les flagelles parasites de quelques insects et sur les infections qu'ils peuvent produire chez les souris. Bull. Soc. Path. Exot. 12: 665-671.

12. Patton, W. S.

1921. Studies on the flagellates of the genera Herpetomonas, Crithidia, and Rhynchoidomonas. No. 7. Ind. Jour. Med. Res. 9: 230-239.

Observations on the Morphology and Life Cycle of Crithidia gerridis Patton in the Water-strider, Gerris remigis Say

This paper is a contribution to the morphology and life cycle of Crithidia gerridis Patton in water striders. Two investigators, Patton and Porter, have already published reports on this subject. Patton (1908) while working in India was the first to describe flagellates from the Heteropteran bugs, Gerris fossarum, Microvelia sp., and Perittopus sp. Porter (1909) studied the morphology and life history of a similar flagellate found in the British species, Gerris paludum, and Fantham recently reported the presence of a crithidia in the water-striders of South Africa. The writer has recently examined various species of North American water-striders, and has found instances of infection of the digestive tract by flagellates belonging to the genus Crithidia in the following species: Gerris remigis, Gerris marginatus, Gerris rufoscutellatus, and Microvelia americana.*

Minchin and Thompson (1915) found that the various stages of Trypanosoma lewisi in the rat flea bore a definite relationship to the various parts of the intestine, and were

* I am indebted to Dr. H. M. Parshley for identifying these species of insects for me.

able to follow chronologically the development in the intestine of the invertebrate host. McCulloch (1919) found that the life cycle of Crithidia euryophthalmi, parasitic in Euryophthalmus convivus, "can be correlated advantageously with the life history of T. lewisi in the invertebrate host, the flea." In my investigation of Crithidia gerridis an attempt has been made to learn how closely its life history corresponds to that of the above mentioned flagellates. The host Gerris remigis was selected for use because it was the most easily obtainable in large numbers.

An average specimen of Gerris remigis, measuring about 1.5 cm. from the vertex of the head to the tip of the abdomen, has a digestive tract from 2.5 to 3.0 cm. in length. Its general gross structure is represented in fig. 1. The esophagus is a thin-walled narrow tube, joining the first stomach with its wall of tall glandular epithelial cells. The long, narrow second stomach is much folded, and may present certain temporary enlargements and constrictions in some specimens. Posteriorly it forms a short bell-shaped enlargement which we may designate as the third stomach. This unites broadly with the ileum into which open the four long and much entangled Malpighian tubules, two from each side. A slight constriction separates the ileum from the rectum, which when empty appears as a white thin-walled

sac. No gastric caeca are present.*

Technique Employed

The living parasites were studied in vaseline sealted coverslip preparations of the various parts of infected intestines teased up in 0.7 per cent. sodium chloride solution. Intra-vitam staining with neutral red, methylene blue, and Janus green was attempted, the last only proving valuable. Methyl green distorted the unfixed flagellates badly, but brought out very well the structure of the nucleus.

For permanent preparations I employed Heidenhain's iron-haematoxylin staining after fixation in Flemming's solution, Bouin's picro-formol, or Schaudinn's fluid heated to about 60° C. Bouin's fluid proved the most valuable fixative. The Giemsa method of staining after fixation in osmic acid vapor gave beautiful pictures, but is not so valuable for critical morphological work. Entire infected intestines were fixed in hot Schaudinn's, sectioned, and stained with Delafield's haematoxylin.

For mitochondrial studies Janus green made up in the proportion of one part of Janus green to 20,000 parts Ringer's solution was used. Permanent preparations were made by the Benda and Bensley methods.

Attempts to culture the Crithidia in Novy and MacNeal,

* The terminology used in this description was adapted to the gut of Gerris remigis from that employed by Glasgow (1914) in his work on The Gastric Caeca and the Caecal Bacteria of the Heteroptera.

Wenyon's, and N.N.N. media failed, probably because of inability to eliminate the growth of bacteria which is fatal to the growth of all the haemoflagellates.

Percentage of Infection

An examination of a number of wild specimens of Gerris remigis disclosed the fact that the infection may be either extremely heavy, where the intestine is actually gorged with parasites, or so light that it is possible to demonstrate the presence of only two or three flagellates. There is always the possibility of overlooking very light infections. However, a record of the examination of two hundred water-striders collected in July, 1921, from a small pond near the fish hatchery at Cold Spring Harbor, Long Island, showed a percentage of infection of 20.5. During April, 1922, 257 water-striders were collected in the vicinity of Baltimore: and of these only six, or 2.5 per cent. were infected. The writer's experience is that the infection rate at other times of the year is about the same in this vicinity.

Distribution in the Host

The esophagus is usually free of flagellates. Except in specimens which have recently fed, the anterior end of the

first stomach is also usually empty. It is in the posterior half of the first stomach where one usually finds the greatest numbers of parasites. Posterior to the first stomach the infection is lighter.

Forms from the First Stomach

McCulloch (1919) following the terminology of Minchin and Thompson (1915) has applied the name nectomonads to the free-swimming flagellated forms of the Crithidia in the insect's intestine, and the name haptomonads to those forms which have attached themselves. In the first stomach of infected Gerris remigis both the free-swimming and the attached form of the parasite may be found, and we shall designate such forms as nectomonads and haptomonads respectively.

Nectomonads (fig. 2-9). Ordinarily these forms predominate in the first stomach. When the gut is ruptured they pour out in great profusion, almost emptying the gut. Some of these long, slender forms swim with great rapidity; they represent the stage of maximum activity in the life cycle of the parasite. Porter has given a good description of their movements. Others are attached together in great agglomeration rosettes as described by Patton and Porter.

A series of 100 measurements showed that these nectomonads range in length from 18.2 u to 60.0 u, and in width from 0.9 u to 6.2 u. The average width was 2.0 u, the average distance from the posterior tip to the nucleus 11.2 u, average distance from the nucleus to the parabasal body 1.9 u, average distance from parabasal body to the anterior tip of flagellum 21.3 u, and the average total length was 34.3 u. I have seen only two individuals in which the parabasal body was posterior to the nucleus (fig. 9). The table (See table I) of 100 measurements of ten flagellates from each of ten insect hosts shows the amount of variation in the size and shape of the flagellate in various hosts of the same species.

Endoplasm. The body is surrounded by an extremely thin periplast which encloses the endoplasm and certain permanent cell structures or organelles. In the fixed and stained preparations the endoplasm takes the appearance of a clear fluid supporting many fine granules. In the living condition, however, these smaller granules are not apparent, but instead one may find larger refractile granular bodies supported in a clear fluid medium (fig. 10). These granules, or "chromidia" as they have been called, seem not to be definitely crystalline in

Table I.

Measurements, in Microns, of 100 Nectomonads Taken 10 Each from 10 Water-Striders

Number of individuals measured	Average of greatest width	Average distance from posterior end to nucleus	Average distance from nucleus to parabasal body	Average distance from parabasal body to anterior end	Range in length	Average total length
10	2.2	5.0	1.1	16.8	18.2-25.9	22.8
10	2.2	9.8	2.1	20.8	25.0-40.4	32.8
10	1.4	19.4	2.4	21.4	33.4-54.0	43.2
10	1.8	10.5	2.2	23.2	29.9-45.5	35.9
10	1.8	9.8	1.2	18.1	20.4-39.6	29.1
10	2.5	5.9	1.3	21.1	21.3-34.5	28.3
10	2.1	8.0	1.4	22.6	22.8-40.3	32.0
10	1.9	13.6	2.2	23.9	32.2-47.1	39.6
10	1.3	19.4	2.7	23.8	40.0-60.0	45.9
10	2.5	10.4	1.9	21.4	25.5-43.6	33.9
Average	2.0	11.2	1.9	21.31		34.3

structure, nor do they take the appearance of droplets. Their shape reminds one rather of the appearance of fine particles of broken glass as viewed by the naked eye. When stained with Janus green they range in color from blue to red-brown. Either they disappear in the process of making fixed and stained preparations, or they are so greatly disintegrated that they appear as the groups of granules shown posterior to the nucleus in the figures.

While studying the "chromidia" of fresh preparations in Janus green with the higher powers of the microscope, I have occasionally seen posterior to the nucleus a rather indistinct structure (fig. 10), if indeed it is a structure. It is rather difficult to distinguish, but at times there seem to be two clear spirally wound streams of fluid which are in continual agitation, due perhaps to the violent lashings of the flagellum. This structure cannot be found in fixed and stained preparations. Perhaps it is merely a streaming appearance of the endoplasm due to osmotic phenomena. Swellengrevel (1911) has figured an axial uncolored spirally wound line in his Giemsa preparations of Herpetomonas calliphorae, which may be homologous with the appearance I have noted in Crithidia gerridis. It seems quite probable that this structure is the rhizostyle of Alexeieff.

Flagellum. As may be seen in Table I the average distance from the parabasal body to the tip of the flagellum, which is in reality the length of the flagellum, averages about 21.31 microns, a fairly constant measurement as compared with other measurements; e.g., the distance from the posterior tip of the cell to the nucleus. The flagellum seems to originate directly from the parabasal body and runs anteriorly just beneath the crest of a thin expansion of the periplast, forming the undulating membrane. Porter, McCulloch and others mention a basal granule or blepharoplast in their crithidias, but I have been unable to demonstrate the like in this particular Crithidia. The basal granule probably lies embedded within the parabasal body, for the flagellum can in almost all instances be traced until it makes a contact with the parabasal body. Very occasionally the flagellum is slightly thickened at the point of juncture with the parabasal body, but no discrete basal granule can be seen (fig. 5.). As further evidence for the invisibility of the basal granule it is worth while to note here that in his account of Tabanus hirtus Patton (1909) makes the statement, "I have never seen a basal granule." Similarly, Swingle (1909) wrote of Crithidia melophagi, "I have never been able to see a granule at the base of the

flagellum and separate from the kinetonucleus."

Parabasal Body. In the unstained preparations this appears as a refractile rod-like body at the base of the flagellum from 1.0 u to 3.2 u in front of the nucleus. It may be either almost perpendicular to the intra-cytoplasmic portion of the flagellum, or inclined at a more or less acute angle. When the parabasal body in fresh preparations is stained with Janus green it seems to lie inside an area of clear fluid, proving that it is not an artefact due to fixation. Various authors have mentioned a definite membrane enclosing this clear area, but neither in the stained nor unstained preparations can this be demonstrated, although the granules of the surrounding protoplasm are often grouped about the edge of this clear area so as to appear somewhat like a membrane in some cases (figs. 5 and 6). The earlier writers, following Woodcock, referred to this structure as the kinetonucleus - hence the order binucleata of Hartmann. Kofoid (1916), and Swezy (1916) have advanced very cogent arguments to prove that it is not a nucleus, and consequently the order binucleata has no justification. They substitute for kinetonucleus the term parabasal body, proposed by Janicki in 1911. Wenyon, Dobell, and Alexeieff have ceased to use the term kinetonucleus, substituting the name kinetoplast. Wenyon (1920)

uses the term kinetoplast to include the parabasal body and blepharoplast or basal granule. Since the name parabasal body was proposed earlier than the name kinetoplast, there is no valid reason for rejecting it and substituting the latter name which may have more significance, especially since in the present state of our knowledge we are not sure that this structure is a plastid. Kofoid (1916) has suggested that the parabasal body is reserve material, and that its formation is the result of a change from the free living to a parasitic mode of life. Alexeieff (1917), however, has advanced some good reasons why this structure is an organ of glycoplastic function, and that it serves to elaborate certain materials of importance in the activity of the flagellate. As was indicated previously, in Crithidia gerridis this body stains with Janus green after the fashion characteristic of mitochondria, but we are by no means certain that as Guilliermond (1918) asserts "The mitochondria give rise to the greater part of the secretory products of the cell and are entirely similar to well known plastids." The opposition of Dangeard (1918) to such views is significant here.

That the chemical nature of the parabasal body is not identical with that of the nucleus is easy to establish, for the nucleus takes little or none of the Janus green stain,

while it is quite pronounced in the parabasal body. Patton (1909) noticed that in the case of the Crithidia from Tabanus hilarius "the nucleus is a circular mass which stains light pink with Romanowsky stain ..... The blepharoplast (parabasal) is a large rod-shaped mass staining deep magenta." Shipley (1916) discovered that the parabasal body and certain granules in the cytoplasm of Trypanosoma lewisi could be stained with Janus green, and that the nucleus remained unstained. The nucleus lies just posterior to the parabasal body, and is usually but slightly less in diameter than the width of the cell at that point. Preparations fixed in Bouin's or Schaudinn's fluid show fairly uniformly a "vesicular" type of nucleus; i.e., there is a deeply staining central karyosome, usually almost round (fig. 3, 4) but sometimes elongated (fig. 2, 8). Infrequently the karyosome is composed of two or three fragments. Surrounding the karyosome is a light space finely and rather diffusely granuled, and the whole is enclosed in a round or oval achromatic "membrane." Sometimes this nuclear "membrane" consists of a number of large clouded granules lying around the periphery (fig. 3). In such cases the name "membrane" may be misleading, because its constituent parts do not seem to form a continuous enclosure. In general, the nucleus does not differ essentially from that described by McCulloch for Crithidia euryophthalmi.

There is a difference, however, between the nucleus of the flagellate found in Gerris remigis, and that described in similar flagellates by Patton and Porter. Patton describes eight chromosomes arranged along the circumference of the nucleus. Porter states, "Large chromatin granules, occasionally eight in number, are present. Usually the chromatin of the nucleus is arranged in slightly irregular masses, etc." Whether these authors relied mainly upon Romanowsky stains of the dry smears , or worked with a different species of Crithidia is uncertain. Swellengrebel (1911) described the nucleus of Crithidia calliphorae as staining deeply, without any differentiation, and surrounded by a clear halo, due to the contraction of the nuclear substance. He adds, "Similar figures have been mistaken for a nucleus containing a large karyosome, and clearly show how defective is this method of fixing and staining," meaning fixation with corrosive alcohol and staining with iron-haematoxylin. It is exactly this picture which one gets with fixation by Schaudinn's, Bouin's, osmic acid (though not so clearly) and staining with iron-haematoxylin, or by the Benda method. Sometimes the living specimen in Janus green shows, somewhat faintly to be sure, the "vesicular" structure of the nucleus. Staining the fresh cells with methyl green had at least one feature of importance; it caused the nucleus to stand out boldly. showing both the karyosome and the nuclear membrane.

Other structures: McCulloch (1917) discovered in Crithidia euryophthalmi a cone shaped structure (parabasal rhizoplast) between the basal granule and the parabasal, and a rhizoplast connecting the nucleus with the basal granule. Since in Crithidia gerridis the flagellum seems to take its origin directly from the parabasal body, there can be found no structure comparable to a parabasal rhizoplast. Very rarely one finds a specimen showing a fine line between the nucleus and the parabasal body, which reminds one of the nuclear rhizoplast seen by McCulloch (fig. 4).

Haptomonads: These are the attached forms which sometimes line the wall of the first stomach in great profusion. They must be grouped into two classes:

(a) Those which are similar morphologically to the nectomonads, except that they are smaller in some cases.

(b) Those which have the same general structure of the organelles as the nectomonads but differ in size and shape (fig. 11, 12, 13, 14). They vary in length from 7.4 u to 25.0 u. and in width from 2.0 to 3.3 u. - somewhat wider than the nectomonad forms. The posterior end is rounded, giving the flagellate an appearance quite different from that of the long graceful nectomonads. These forms attach themselves in great masses to the tips and sides of the tall epithelial cells.

Cyst-like forms. Patton and Porter mention finding cysts in the crop of the insects, and they describe a process of development from the cyst to the flagellate stage. Almost any interpretation can be placed upon dried preparations of the haptomonad forms stained by the Romanowsky method, since some show either no flagellum or a faintly staining one. Iron-haematoxylin preparations of these same forms show a well-develcped flagellum in all instances. I believe that most of the cyst forms which Patton and Porter found in the crop were in reality degenerated haptomonads or yeasts.

Second stomach, third stomach, ileum. The third stomach and ileum rarely contain more than a comparatively insignificant number of flagellates. In some heavy infections of the first and second stomach, these sections of the intestine will contain at most only a few nectomonads swimming in their contents and a few haptomonads attached to the intestinal wall. The anterior end of the first stomach, however, often swarms with flagellates which resemble those in the posterior portion of the first stomach. Generally speaking, the infection becomes scantier as one proceeds backward from the anterior end of the first stomach toward the rectum.

Rectum. Minchin and Thompson (1915) found that Trypanosoma lewisi might permanently establish itself in the rectum of the rat flea after the infection had disappeared

from the anterior portions of the intestine. McCulloch (1913) found that there was a permanently established phase of the life cycle of the parasite in the pyloric expansion of the lupine bug corresponding to the established rectal phase of Trypanosoma lewisi. In the water-strider one finds few flagellates attached to the wall of the rectum, so that it seems improbable that rectal haptomonads are a part of the life cycle. However, a few haptomonads (fig. 14) and nectomonads are present which have probably passed down from the anterior part of the intestine.

The rectum often contains another form of the parasite which is occasionally encountered anteriorly. It is a long free-swimming form, very narrow 0.7 u to 1.2 u in width, with a short body and a long flagellum, making the total length from 21 u to 38 u (fig. 17, 18, 19). On account of their small size it was impossible to study accurately their nuclear structure. The "spirochaetiform" flagellates which Porter figures from the rectum resemble these in that they are very narrow, but the cell proportions are quite different.

The structure of this type of flagellate is remarkedly like that of Herpetomonas. It is too small to determine whether or not there is a rudimentary membrane. That it is a stage in the life history of this Crithidia is certain, however, for no

infections are to be found where there are not also crithidial forms present, and all gradations of the flagellate between this type and the large flagellates may be found. Here also are found the encysting and cyst forms already described by Porter (fig. 20, 21, 22). The cysts measure from 1.5 to 4.0 microns in diameter. A definite cyst wall is lacking. Whether or not this is the infective stage has never been experimentally proved under conditions which would eliminate the presence of all other forms of the parasite. Wenyon (1912) was able to cultivate herpetomonads from the faeces of Pulex irritans which had been allowed to dry for 24 hours. This might be taken as evidence that these rounded forms are the actual resistant infective forms.

Manner of Multiplication

No method of multiple fission corresponding to the multiplication spheres of Minchin and Thompson, or to the somatellas of McCulloch was found. Sections of two entire heavily infected intestines and study under the microscope of scores of fresh intestines failed to disclose intracellular phases of any nature in the life history. The very interesting process of endogenous budding in Crithidia euryophthalmi by McCulloch was carefully searched for with

negative results. Appearances simulating the multiplication rosettes of Patton and Porter were found, but this method certainly does not play any prominent part in the multiplication of the parasites.

The prevailing method of multiplication of all types of crithidias present in an infection is binary division. Only a relatively few specimens in the advanced stages of division are to be found, but a large proportion of the number in one stained smear will show the beginning processes of division.

The first apparent indication of division is a dividing parabasal body (fig. 5, 14, 24). The two halves move away from each other, connected at first by a fine filament (fig. 6, 7, 25, 26). Apparently the old flagellum remains with the half to which it is attached, and the new flagellum arises from the other half. Whether the flagellum divides or splits has been the subject of much controversy. The new flagellum is so precocious in its growth, and follows so closely the course of the old one that it is difficult to follow the process exactly. Such appearances as in fig. 14 suggest that the new flagellum is a new outgrowth.

Meanwhile the karyosome of the nucleus commences to lengthen in the direction of the long axis of the cell (fig. 5, 24, 25). Next it swings to an angle, sometimes taking a position at an angle of 90° to its former position (fig. 6, 7, 26).

A constriction appears at about the middle of the elongated karyosome as the two halves move away from each other (fig. 6, 7, 26, 27). At this stage the nuclear membrane becomes indistinct, the clear area surrounding the karyosome diminishes so that the karyosome seems to lie almost in contact with the granular endoplasm (fig. 7, 26, 27).

While the nuclear division is advancing to this stage the body of the flagellate widens quite noticeably in the vicinity of the nucleus. A clear groove interposes between the members of the pair in process of development, across which a dark staining filament connecting the two members of the dividing karyosome may run (fig. 7, 26, 27). As this groove widens it extends anteriorly until both anterior ends are free (fig. 28). Usually before the division posteriorly is complete the two individuals have their nuclei and undulating membranes completely reconstructed. The two flagellates then pull apart posteriorly.

There is no indication that the granules posterior to the nucleus ("chromidia") divide. They go passively with the half of the body in which they are embedded.

In the division of the nucleus there is no indication of a centrosome or attraction sphere. The karyosome seems to supply the activity needed for a nuclear division. If we regard the karyosome as the active element in the division we may consider the process one of promitosis.

Summary and Conclusions

1. The North American species of water-strider, Gerris remigis, was found to be parasitized by a flagellate belonging to the genus Crithidia. The infection rate at Cold Spring Harbor was 20.5 per cent; in the vicinity of Baltimore, 2.5 per cent.
2. The infections studied ranged from extremely light to the condition where the intestine is filled with the parasites. The parasites may be found in the intestine from the first stomach to the rectum.
3. In the first stomach are found the nectomonads, or free-swimming forms, and the haptomonads, or attached forms.
    a. Measurements of 100 nectomonads are tabulated, and the endoplasm and organelles described.
    b. The endoplasm contains granules which can be stained with Janus green. In the endoplasm of the living cell posterior to the nucleus a rather indistinct structure having the appearance of two clear streams of protoplasm is visible.
    c. The flagellum apparently arises directly from the parabasal body. No discrete basal granule can be distinguished.

d. The parabasal body lies in a clear area which is not surrounded by a definite membrane. It stains with Janus green, which shows that it is mitochondrial in nature.

e. The nucleus is "vesicular" in structure: - there is a central karyosome surrounded by an achromatic membrane.

f. Occasionally a fine line between the nucleus and parabasal body can be seen. It in homologous to the parabasal rhizoplast of McCulloch.

g. The morphology of the haptomonads is essentially the same as that of the nectomonads, except that the former are shorter and the posterior end is wider and more rounded.

3. The second stomach, third stomach, and ileum may have all the forms found in the first stomach, but the number of parasites in these portions of the intestine is much less.

4. In the rectum are found the following forms:

a. Nectomonads from anterior part of intestine.

b. Haptomonads from anterior part of intestine.

c. A very narrow free-swimming nectomonad form with a long flagellum is the predominating type.

d. The cysts and encysting forms.

5. The only process of multiplication observed was binary fission. No "somatellas," "tailed spheres," or intracellular stages were observed in the life history of this Crithidia.

6. The finding of two flagellates with the parabasal bodies located posterior to the nucleus demonstrates further the close relationship of these flagellates to the trypanosomes.

Bibliography

1. Alexeieff, A. 1917. Sur les mitochondries a fonction glycoplastique. Compt. rend. Soc. d. Biol., Paris, 80: 510-512.

2. Dangeard, P. A. 1918. Sur la nature du chondriome et son role dans la cellule. Compt. rend. Acad. Sci., 166: 439-446.

3. Glasgow, Hugh. 1914. The gastric caeca and the caecal bacteria of the Heteroptera. Biol. Bull., 26: 101-170.

4. Guilliermond, A. 1918. Sur la nature et la signification du chondriome. Compt. rend. Acad. Sci., 166: 649-651.

5. Kofoid, C. A. 1916. The biological and medical significance of the intestinal flagellates. Proc. Soc. Pan-Amer. Sci. Congress, 1915-1916.

6. McCulloch, Irene. 1917. Crithidia euryophthalmi, sp. nov. from the Hemipteran Bug, Euryophthalmus convivus Stal. Univ. Calif. Publ. Zool., 18: 75-88.

7. McCulloch, Irene. 1919. A comparison of the life cycle of Crithidia with that of Trypanosoma in the invertebrate host. Univ. Calif. Publ. Zool., 19: 135-190.

8. Minchin, E. A., and Thompson, J. P. 1915. The rat trypanosome, Trypanosoma lewisi, and its relation to the rat flea, Ceratophyllus fasciatus. Quart. Jour. Micro. Sci., 60 (N.S.): 463-692.

9. Patton, W. S. 1908. The life cycle of a species of Crithidia parasitic in the intestinal tract of Gerris fossarum Fabr. Arch. f. Prot., 12: 131-146.

10. Patton, W. S. 1909. The life cycle of a species of Crithidia parasitic in the intestinal tracts of Tabanus hilarius and Tabanus sp. Arch. f. Prot., 15: 333-362.

11. Porter, Annie, 1909. The morphology and life history of Crithidia gerridis, as found in the British water bug, Gerris paludum. Parasit., 2: 348-366.

12. Shipley, P. G. 1916. The vital staining of mitochondria in T. lewisi with Janus green. Anat. Rec., 10: 439-445.

13. Swellengrebel, N. H. 1911. Note on the morphology of Herpetomonas and Crithidia. Parasit., 4: 108-130.

14. Swezy, Olive. 1916. The kinetonucleus of flagellates and the binuclear theory of Hartmann. Univ. Calif. Publ. Zool., 15: 185-240.

15. Swingle, L. D. 1909. A study on the life history of a flagellate in the alimentary tract of the sheep tick. Jour. Inf.

Dis., 6: 98-121.

16. Wenyon, C. M. 1912. Observations on the behavior of Leishmania tropica and allied flagellates in bugs and fleas. Journ. Lond. Schl. Trop. Med., 2: 13-26.

17. Wenyon, C. M. 1920. Observations on the intestinal protozoa of three Egyptian lizards, etc. Parasit., 12, 350-365.

Fig. 1, diagrammatic drawing of the digestive tract of Gerris remigis. e, esophagus; 1, first stomach; 2, second stomach; 3, third stomach; i, ileum; r, rectum.

x 2140

Fig. 2, large nectomonad, type commonly found in first and second stomachs.

Fig. 3, nectomonad with nuclear membrane composed of a ring of clouded granules.

Fig. 4, nectomonad with an apparent nuclear rhizoplast, undulating membrane only slightly developed.

Fig. 5, nectomonad commencing division.

Fig. 6, a further division stage.

Fig. 7, the parabasal body and the nucleus are still incompletely divided and the cytoplasm has commenced to split between the two new individuals in this advanced division stage.

Fig. 8, a nectomonad with a well developed undulating membrane, and incidentally, the posterior end is club-shaped.

Fig. 9, a trypanomorphic Crithidia, the parabasal body lies posterior to the nucleus.

Fig. 10, a purely schematic drawing of a nectomonad showing cell structure as it appears after vital staining with Janus green.

x 2140

Figs. 12, 13, 14, haptomonads from the first stomach.

Fig. 15, a haptomonad form found in the rectum, showing a dividing parabasal body and the new flagellum beginning to grow out from the parabasal body.

Fig. 16, 17, 18. Small nectomonad forms with long flagella from the rectum.

Fig. 19, an encysting form.

Figs. 20, 21. The so-called cyst forms.

Fig. 22. Outline drawing of cross section of intestine showing haptomonads attached to the intestinal wall.

Fig. 23-28. Outline drawings of dividing forms arranged in series so as to show the process of division.

Biography

Elery Donald Becker. Born near Sterling, Illinois, December 5, 1896. Education - Elementary education received in the public schools of Sterling, Illinois. Completed a four-year course in Rocky Ford High School, Colorado, June, 1915. Received the Bachelor of Arts degree from the University of Colorado, June, 1921, in absentia. Commenced graduate work in the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, October 1920. At present time, a graduate student and assistant in protozoology.